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13. ABSTRACT *(Maximum 200 words)*

During the past contract year, studies were initiated to determine the pharmacokinetics and metabolism of [^{14}C]artelinic acid in male and female dogs, and to investigate the dose-related pharmacodynamic effects of artelinic acid, as assessed by signs of clinical and pathological toxicity, in male and female rats. Results of the pharmacokinetic study indicate that in dogs: the absorption of radioactivity derived from [^{14}C]artelinic acid is relatively rapid after oral administration of the compound; after either IV or PO administration of [^{14}C]artelinic acid, radioactivity is detectable in whole blood and plasma for greater than 192 hours after dosing; [^{14}C]artelinic acid and/or its radiolabeled metabolites appear to undergo biliary excretion; artelinic acid is extensively metabolized. The identity of the metabolites of artelinic acid is currently under investigation. The initial phase of the pharmacodynamic study of artelinic acid in rats is currently in progress and, thus, definitive information is not yet available from this study.

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Patricia E. Noker 9/10/99

Patricia E. Noker, Ph.D., Principal Investigator Date

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I. INTRODUCTION

The work scope of this contract involves the performance of studies in rats and dogs on the pharmacokinetic and pharmacodynamic properties of drugs under clinical development by the U.S. Army Medical Research and Development Command. The pharmacokinetic aspect of these studies involves an investigation of the absorption, disposition, metabolism (biotransformation) and elimination of test compounds in experimental animals. The pharmacodynamic aspect involves relating certain measured parameters, for example, the production of methemoglobin, to blood and plasma levels of the test compound and/or its metabolites, or assessing toxicological parameters such as clinical signs and mortality occurring after administration of a test compound. The information derived these studies is intended to provide a data base for establishing an appropriate species and appropriate doses for subsequent subchronic and chronic toxicity studies, to predict for possible organ toxicities which might occur, and to generate data required by the Food and Drug Administration prior to submission of a Notice of Claimed Investigational Exemption for a New Drug (IND) and New Drug Applications, Human Use (NDA).

During the past year of the contract, pharmacokinetic/pharmacodynamic studies were initiated with the anti-malarial agent, artelinic acid. Information relevant to USAMRDC's interest in developing artelinic acid for clinical use is provided below.

Early in this century, with the discovery of quinine, malaria appeared to be one of the few diseases for which a specific cure existed. As a result, however, of the development of insecticide resistance by the *Anopheles* mosquito and the development by this parasite of resistance to chloroquine and mefloquine, malaria remains a major health problem in many areas of the world.[1] Qinghaosu (QHS), also known as artemisinin, was initially isolated and characterized by the Chinese and found to be an effective antimalarial agent.[2] This compound is a sesquiterpene lactone with an endo-peroxide bridge. The methyl ether (artemether) of reduced QHS, dihydroqinghaosu (DQHS), and the succinate hemiester of DQHS, artesunic acid, are also effective in the treatment of severe cases of multi-drug resistant malaria.[3] These compounds, while therapeutically active, have several limitations, one of which is their thermal lability.

In the search for better antimalarial agents, artelinic acid, a semisynthetic water-soluble derivative of QHS, was developed. Artelinic acid, prepared as the sodium salt, has been reported to be effective against chloroquine-resistant *P. falciparum* and to be superior to QHS against *P. berghei*.[4] The compound has also been reported to be 500 to 1000-fold more stable than artesunic acid when maintained at comparable temperature and pH conditions.[5] Artelinic acid is, therefore, of potential value in the treatment of drug-resistant malaria.

The toxicity of QHS and its derivatives is reportedly low.[6] However, recent evidence has indicated that high-dose arteether produces a distinctive and progressive syndrome of neurological deficits in rats, dogs, and rhesus monkeys.[6,7] Histopathological data indicate that the brainstem is selectively affected. There is a concern, therefore, that artelinic acid may produce a similar neuropathy.

Only limited information is available on the pharmacokinetics, bioavailability, and metabolism of artelinic acid, and other derivatives of QHS, in experimental animals. Li et al conducted a comparative investigation of the pharmacokinetics and bioavailability of DQHS, arteether, artemether, artesunic acid, and artelinic acid, following IV, IM, and PO doses of 10 mg/kg to rats.[8] The results of this study indicated that highest plasma drug levels were achieved with artelinic acid, presumably because of its relatively low degree of metabolism to DQHS and slow rate of elimination. In our laboratory, the pharmacokinetics of [^{14}C]artelinic acid were investigated following IV or PO administration to rats [9]. It was determined that, whereas the oral bioavailability of radioactivity derived from [^{14}C]artelinic acid was approximately 70%, the oral bioavailability of unchanged artelinic acid was only approximately 28%. Following either IV or PO administration of [^{14}C]artelinic acid to rats, approximately 40% of the dose was eliminated in urine and 55% in feces within 192 hours of dose administration. Li and coworkers have also determined the pharmacokinetics of artelinic acid in dogs after IV, IM, or PO administration.[10] Their results indicated that artelinic acid was eliminated in two phases after each of these routes of administration with half-lives of 0.37-0.54 hours and 2.21-6.14 hours. Further, the bioavailability of artelinic acid after IM administration was determined to be 90% and, after PO administration, 80%.

In order to gain additional information on the pharmacokinetics and metabolism of artelinic acid in experimental animals and to assess the potential of artelinic acid to produce neuropathological lesions, the following studies were initiated during the past year of this contract:

- Task Order SR98-2: Bioavailability, Pharmacokinetics, and Identification of Metabolites of [^{14}C]Artelinic Acid in Dogs.

  The objectives of this study are to investigate, in both male and female beagle dogs, the time concentration profiles of artelinic acid and its metabolites in whole blood, plasma, urine, and feces following IV and PO administration of [^{14}C]artelinic acid; to determine the oral bioavailability of artelinic acid; and to elucidate the identity of the major metabolites of artelinic acid.

  To this end, dogs were administered a single IV or PO dose of [^{14}C]artelinic acid. Blood/plasma, urine, and feces were then collected from each animal at various times through 192 hours after dosing and submitted for analysis for total radioactivity, unchanged parent compound, and metabolites.

- Task Order SR98-3: Effect of Artelinic Acid on Rats after Oral Administration for 14 Days.

  The objective of this study is to determine the dose-related pharmacodynamic effects of artelinic acid, as assessed by signs of clinical and pathological toxicity, in rats following daily administration by oral gavage for 14 consecutive days. This includes determination of a no-effect dose level, target organ toxicity, and the reversibility of target organ toxicity.

The study includes two phases: an initial dose range finding evaluation to select for an appropriate dose(s) of artelinic acid that produces a measurable pharmacodynamic effect; and a subsequent definitive study to fully characterize any dose-related pharmacodynamic effects of artelinic acid. In each phase of the study, a positive control group, that receives arteether, is also included. Assessments include: examinations of individual animals for clinical signs of toxicity, body weight measurements, and histopathological evaluations of tissues.

II. METHODS

A. Test Compounds

Artelinic acid [4-(10'-dihydroartemisininoxymethyl)benzoic acid] was synthesized by Starks Associates, Inc. (Buffalo, NY) and supplied by Walter Reed Army Institute of Research (Washington, DC). [16-^{14}C]artelinic acid was synthesized by Research Triangle Institute (Research Triangle Park, NC). The stated specific activity and radiochemical purity of the [^{14}C]artelinic acid were 26.2 μCi/mg and 97%, respectively. The radiochemical purity of the compound was verified at Southern Research upon receipt.

Arteether was synthesized by Starks Associates, Inc. (Buffalo, NY) and supplied by Walter Reed Army Institute of Research (Washington, DC).

B. Test Compound Formulation

1. For the study in dogs

The dose formulation of artelinic acid for IV administration was prepared to contain approximately 10 mg of artelinic acid per mL and to have a radioactivity content of approximately 20 μCi/mL. For preparation, calculated quantities of unlabeled artelinic acid and [^{14}C]artelinic acid were dissolved in 5% sodium carbonate in sterile water. The resulting solution was then diluted to the desired volume with 0.9% saline.

The dose formulation for PO administration was prepared to contain approximately 5 mg of artelinic acid per mL and to have a radioactivity content of approximately 10μCi/mL. For preparation, calculated quantities of unlabeled artelinic acid and [^{14}C]artelinic acid were suspended in 1% carboxymethylcellulose (CMC):0.5% Tween 80.

2. For the study in rats

Dose formulations of artelinic acid were prepared to contain either 2, 4, 8, 16, or 64 mg/mL of artelinic acid in 1% CMC:0.2% Tween 80. For preparation of each formulation, a calculated

quantity of artelinic acid was added to 1% CMC:0.2% Tween 80 and then mixed by homogenizing until a visually uniform suspension was obtained.

A dose formulation of arteether was prepared to contain 25 mg/mL in sesame oil. For preparation, a calculated quantity of arteether was added to sesame and mixed by stirring until solution of the compound was obtained.

C. Dose Formulation Analysis

Upon preparation, each unlabeled dose formulation was assayed for chemical concentration and each radiolabeled formulation was assayed for chemical concentration, total radioactivity, and radiochemical purity.

Chemical concentration determinations on dose formulations of artelinic acid were accomplished by HPLC using a Bondapak C18 (10 μm) column and a mobile phase of 65% methanol:35% 0.1 M aqueous ammonium acetate at a flow rate of 1.5 mL/min. Detection was by UV absorbance at 235 nm.

Chemical concentration determinations on the dose formulation of arteether were accomplished by HPLC using a C18 (10 μm) column and a mobile phase of 60% acetonitrile:40% water at a flow rate of 1.5 mL/min. Detection was by UV absorbance at 216 nm.

Total radioactivity determinations were made by direct radioanalysis of appropriate dilutions, prepared in triplicate, of each radiolabeled dose formulation. Radiochemical purity was verified by HPLC analysis using an in-line Flo-One/Beta Series A-500 radiochromatography detector (Radiomatic Instruments and Chemical Company, Inc., Meriden, CT).

D. Test Animals

Male and female dogs were purchased from Covance Research Products, Inc. (Cumberland, VA). Dogs were quarantined for a minimum period of two weeks prior to use in the study. During the quarantine period, each animal was given a complete physical examination which included hematological and clinical chemistry determinations. Body weights and rectal temperatures were monitored. Dogs were fed Certified Canine Diet #5007 (PMI Feeds, Inc., St Louis, MO) and given city tap water. The dogs were individually housed in stainless steel cages during both the quarantine and experimental periods. Each dog was identified by an ear tattoo. Dogs were approximately 8-12 months old at the beginning of each study and weighed between 7 and 11 kg. Four male and four female dogs were used during the study.

Male and female Sprague Dawley rats were purchased from Charles River Laboratories (Raleigh, NC). Animals were quarantined for a minimum period of one week prior to use. Rats were group housed in polycarbonate cages lined with hardwood chips. Certified Rodent Diet #5002, PMI Feeds, Inc.) and city tap water were provided to the animals *ad libitum*.

All animal care and housing was conducted in accordance with the guidelines specified in the *Guide for Care and Use of Laboratory Animals*, 7th Edition (Institute of Laboratory Animal Resources, Commission on Life Sciences, National Research Council, National Academy Press, Washington, D.C., 1996), the U.S. Department of Agriculture through the Animal Welfare Act (Public Law 99-198), and current AAALAC regulations.

E. Experimental Procedures

1. Dose administration

IV doses were administered to dogs via a catheter inserted into a peripheral vein; doses were given by slow-push injection over 2-3 minutes. Oral doses were given by gavage using either a gastric tube (dogs) or a round-tipped feeding needle (rats).

2. Measurements

a.) Mortality

Dogs and rats were observed for mortality and morbidity twice a day during the quarantine and study periods.

b.) Clinical observations

Clinical observations were performed on dogs three times on the day of dosing and at least twice daily thereafter for 7 days. For rats, a detailed clinical evaluation of each animal was performed daily on the days of dose administration; this evaluation was performed within approximately 1 hour after dosing. In addition, cage-side observations (for appearance, gait, activity, level of arousal, and motor coordination) were performed on each animal 1-3 hours and 7-9 hours after dosing.

c.) Body weights

Dogs were weighed prior to dosing. Rats were also weighed daily during the period of dose administration.

3. Sample collection

a.) Blood collection

Blood samples were collected from the jugular vein of unanesthetized dogs into tubes containing heparin. Upon collection, a portion of each whole blood sample was removed and saved. The remainder of each sample was then centrifuged to obtain plasma.

b.) Urine and feces collections

Urine and feces were collected from dogs maintained in individual stainless steel metabolism cages at 0-12, 12-24, 24-48, 48-72, 72-96, 96-120, 120-144, 144-168, and 168-192 hours after either IV or PO administration of [^{14}C]artelinic acid. At each excreta collection, each cage pan was rinsed with water and the rinse was saved.

4. Sample Analysis

a.) Equipment

Samples were oxidized using a Packard Model 307 sample oxidizer (Packard Instrument Company, Inc., Downers Grove, IL). Radioactive samples were counted in a Tri-Carb 2100 TR liquid scintillation analyzer (Packard Instrument Company, Inc.). Radioanalyses were corrected for counting efficiency and background radioactivity.

b.) Whole blood and plasma

Duplicate portions of each whole blood and plasma sample were placed in individual oxidizer cones and combusted. The resultant samples were assayed for radioactivity.

c.) Urine, feces, and cage rinses

The volume of each urine and cage rinse sample was obtained. Duplicate portions of each sample were placed in individual oxidizer cones and combusted. The resultant samples were assayed for radioactivity. Fecal samples were weighed and homogenized in 9 volumes of water. Quadruplicate portions of each homogenate were placed in individual oxidizer cones and combusted. The resultant samples were assayed for radioactivity.

Urine and feces samples were also analyzed by HPLC to determine the profile of the radiolabeled sample components. Prior to analysis, urine samples were clarified by centrifugation. Fecal homogenates (prepared in water) were centrifuged after alkalinization with sodium bicarbonate and the resultant supernatant was analyzed by HPLC. The conditions of analysis were as follows:

Column:	Capcell C8, 4.6 mm x 250 mm
Mobile phase A:	0.25% phosphoric acid
Mobile phase B:	50% methanol:20% acetonitrile:30% water containing 0.25% phosphoric acid
Elution:	Gradient

Program:	Min.	Flow mL/min	%A	%B
	Initial	1.0	80	20
	20	1.0	65	35
	40	1.0	55	45
	50	1.0	55	45
	60	1.0	40	60
	70	1.0	0	100
	80	1.0	0	100

Detection: In-line radiodector

5. Data analysis

To calculate the amount of radioactivity present in the total collection of a sample or in a mL of sample, radioassay results were corrected for counting efficiency and background radioactivity and then multiplied by an appropriate factor to correct for sample dilution and/or volume assayed. The specific activity of each dosing solution was calculated by dividing the radioactivity present in each mL of solution (as determined by direct scintillation counting) by the concentration, in mg, of test compound in each mL of solution (as determined by HPLC). Conversion of radioassay results obtained for blood, plasma, urine, and feces to μg equivalents was accomplished by dividing radioactivity amounts in each sample by the specific activity (μCi/mg) of the appropriate dosing solution.

III. RESULTS AND DISCUSSION

A. T.O. SR98-2: Bioavailability, Pharmacokinetics, and Identification of Metabolites of [^{14}C]Artelinic Acid in Dogs

The results of the radioanalyses of the whole blood and plasma samples collected after IV administration of [^{14}C]artelinic acid to two male and two female dogs are shown in Tables 1 and 2, respectively. No sex-related differences were apparent in either whole blood or plasma concentrations of radioactivity among the IV dosed animals. Peak levels of radioactivity in whole blood, ranging from 18.6 to 22.9 μg equivalents/mL, were observed 5 minutes after dosing (earliest time point). The corresponding plasma concentrations of radioactivity at this time were higher and ranged from 30.3 to 36.4 μg equivalents/mL. These data indicated that, at least at the early times after dosing, radioactivity derived from [^{14}C]artelinic acid remained predominantly in the plasma compartment of whole blood and not in the red blood cells. Among the individual dogs, the concentration of radioactivity in blood and plasma declined with time; at 48 hours and subsequent times after dosing, the concentration of radioactivity in blood was similar to that observed in plasma.

For two male and two female dogs administered a PO dose of [^{14}C]artelinic acid, sex-related differences in either whole blood or plasma concentrations of radioactivity were not apparent. Peak concentrations of radioactivity in whole blood and plasma were observed at 30 minutes after dosing (earliest time point) (Tables 3 and 4). At this time, concentrations of radioactivity in whole blood ranged from 5.1 to 7.5 μg equivalents/mL among the four dosed dogs; the corresponding concentrations of radioactivity in plasma ranged from 8.5 to 12.3 μg equivalents/mL. As observed after IV dosing, the concentrations of radioactivity in plasma remained higher than those in whole blood during the first 48 hours after dosing. Beyond this time, the concentrations of radioactivity in whole blood and plasma were similar at the respective points.

A summary of the urinary and fecal elimination of radioactivity by the individual animals given either an IV or PO dose of [^{14}C]artelinic acid is shown in Table 5. For dogs given an IV dose, the mean recovery of the administered dose was 93.2%. Of this amount, a mean value of 37.2%, 8.8%, and 47.3% was recovered in urine, the cage rinse, and feces, respectively. The fecal elimination of radioactivity following IV administration of [^{14}C]artelinic acid indicated that the compound and/or its metabolites underwent biliary excretion. For dogs given a PO dose, the mean recovery of the administered dose was 92.9%. Of this amount, a mean value of 25.5%, 9.9%, and 57.5%, respectively, was recovered in urine, the cage rinses, and feces, respectively.

Representative radiochromatograms obtained during the HPLC analyses of urine samples are shown in Figure 1. The results of HPLC analyses of urine collected 0-12 or 12-24 hours after dosing indicated that the metabolite profile of the radioactivity eliminated in urine was similar after either IV or PO administration of [^{14}C]artelinic acid. Unchanged [^{14}C]artelinic acid was not detectable in urine collected at any time after either IV or PO administration of the compound; at least three major radiolabeled metabolites were resolved (Figure 1).

Representative radiochromatograms obtained during the HPLC analysis of fecal extracts from dogs administered either an IV or PO dose of [^{14}C]artelinic acid are shown in Figures 2 and 3, respectively. The radioactivity profile of fecal samples obtained after IV administration of [^{14}C]artelinic acid was similar to the profile of samples collected after PO administration of the compound. No unchanged artelinic acid was detected in feces. The majority of the radioactivity in the fecal extracts was accounted for by one minor and two major radiolabled metabolites. The elution time of these metabolites was different from that for the metabolites resolved in urine under the same HPLC conditions of analysis.

These results of the analyses of the urine and fecal samples indicated that artelinic acid was extensively metabolized by dogs. Elucidation of the identity of the urinary and fecal metabolites is currently under investigation.

B. T.O. SR98-2: Effect of Artelinic Acid on Rats after Oral Administration for 14 Days

The dose range finding phase of this study was initiated on August 25, 1999 and, thus, the in-life portion of the study is still in progress. This phase of the study involves the administration of either PO doses of artelinic acid or an IM dose of arteether (the positive control) to male and female rats once each day for 14 consecutive days as follows:

Group ID	Compound	Dose Route	mg/kg/day	No. Males	No. Females
V	None[a]	PO	0	2	2
A	Artelinic Acid	PO	10	4	4
B	Artelinic Acid	PO	20	4	4
C	Artelinic Acid	PO	40	4	4
D	Artelinic Acid	PO	80	4	4
E	Artelinic Acid	PO	320	2	2
F	Arteether	IM	12.5	2	2

[a]Rats are being given 1% carboxymethylcellulose and 0.2% Tween 80

Through Day 12 of the study, no clinical signs of toxicity and no mortality have been observed for both male and female rats in Groups V, A, B, C, D, and F, and for male rats in Group E. One female rat in Group E was found dead on the morning of Day 12 of the dosing period. Prior to death, this animal appeared emaciated and hunched (starting on Day 8) and exhibited a nose and eye discharge (starting on Day 9). The other female rat in Group E was found dead on Day 12; this animal also appeared emaciated and hunched, starting on Day 8, prior to death.

Body weight reductions (20-30% body weight loss) have been noted between Day 1 and 12 for the two male and two female rats in Group E.

More definitive information on the pharmacodynamic effects of artelinic acid will not be available until the completion of the histopathological evaluations which are to be performed on tissues collected on Day 21 of the study.

IV. CONCLUSIONS

The data currently available from the pharmacokinetic study with [^{14}C]artelinic acid in dogs indicate that:

Radioactivity derived from [^{14}C]artelinic acid is relatively rapidly absorbed after oral administration of the compound.

After either IV or PO administration of [^{14}C]artelinic acid, radioactivity is detectable in whole blood and plasma for greater than 192 hours after dosing.

In dogs, [^{14}C]artelinic acid and/or its radiolabeled metabolites appear to undergo biliary excretion.

Artelinic acid is extensively metabolized by dogs.

Due to the limited data currently available, no conclusions can be drawn on the pharmacodynamic effects of artelinic acid in rats.

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VI. SIGNATURE PAGE

This report was written by:

Patricia E. Noker

Patricia E. Noker, Ph.D., D.A.B.T.
Principal Investigator

9/10/99
Date

Reviewed by:

Ward R Richter

Ward R. Richter, D.V.M., M.S., D.A.C.V.P.
Director, Safety Assessment Department

9/10/99
Date

Table 1. Levels of Radioactivity in Blood Following IV Administration of [^{14}C]Artelinic Acid to Dogs[a]

Sample Time (hr)	AM2249 ugeq/mL	AM2250 ugeq/mL	AF2253 ugeq/mL	AF2255 ugeq/mL	Mean ugeq/mL	S.D.
0.083	19.1	22.9	21.2	18.6	20.5	2.0
0.25	14.1	16.5	15.9	14.4	15.2	1.1
0.5	9.4	11.5	10.8	9.8	10.4	0.9
1	4.9	6.7	5.7	5.5	5.7	0.8
2	2.6	3.5	2.6	2.5	2.8	0.5
4	2.9	3.6	1.6	1.9	2.5	0.9
8	1.8	1.7	1.2	1.9	1.6	0.3
12	1.6	2.5	1.2	1.3	1.7	0.6
24	0.76	1.3	0.93	0.79	0.95	0.3
48	0.31	0.43	0.41	0.33	0.37	0.1
72	0.22	0.29	0.29	0.23	0.26	0.04
96	0.18	0.25	0.22	0.21	0.21	0.03
120	0.15	0.24	0.21	0.18	0.19	0.04
144	0.15	0.21	0.17	0.17	0.18	0.03
192	0.14	0.18	0.16	0.15	0.16	0.02
Total nCi						

[a] Dogs AM2249 and AM2250 were male and dogs AF2253 and AF2255 were female.

Table 2. Levels of Radioactivity in Plasma following IV Administration of [^{14}C]Artelinic Acid to Dogs[a]

Sample Time (hr)	AM2249 ugeq/mL	AM2250 ugeq/mL	AF2253 ugeq/mL	AF2255 ugeq/mL	Mean ugeq/mL	S.D.
0.083	30.3	36.4	32.3	31.7	32.7	2.6
0.25	22.0	25.3	24.7	23.5	23.9	1.4
0.5	14.8	17.6	16.9	15.3	16.2	1.3
1	7.7	10.1	8.6	8.5	8.7	1.0
2	4.1	5.3	4.0	4.0	4.4	0.6
4	4.6	5.4	2.4	2.8	3.8	1.4
8	3.1	3.6	2.0	3.4	3.0	0.7
12	2.9	3.9	1.8	2.7	2.8	0.9
24	1.2	2.0	1.3	1.2	1.4	0.4
48	0.41	0.52	0.51	0.43	0.47	0.1
72	0.27	0.33	0.34	0.29	0.31	0.03
96	0.21	0.30	0.30	0.26	0.27	0.04
120	0.18	0.25	0.25	0.20	0.22	0.03
144	0.16	0.22	0.20	0.18	0.19	0.02
192	0.12	0.17	0.18	0.15	0.15	0.03

[a]Dogs AM2249 and AM2250 were male and dogs AF2253 and AF2255 were female.

Table 3. Levels of Radioactivity in Blood following PO Administration of [^{14}C]Artelinic Acid to Dogs[a]

Sample Time (hr)	BM2251 ugeq/mL	BM2252 ugeq/mL	BF2254 ugeq/mL	BF2256 ugeq/mL	Mean ugeq/mL	S.D.
0.5	5.1	7.2	7.5	6.7	6.6	1.1
1	4.2	4.4	5.4	4.9	4.7	0.6
2	2.7	2.9	3.5	2.5	2.9	0.4
4	2.8	1.8	2.2	1.7	2.2	0.5
8	1.7	1.2	1.8	1.6	1.6	0.3
12	1.6	1.7	1.4	1.0	1.4	0.3
24	0.73	1.5	0.95	0.57	0.93	0.4
48	0.31	0.40	0.39	0.30	0.35	0.1
72	0.24	0.24	0.34	0.22	0.26	0.1
96	0.22	0.19	0.26	0.17	0.21	0.04
120	0.19	0.17	0.22	0.17	0.19	0.02
144	0.15	0.16	0.19	0.14	0.16	0.02
192	0.15	0.13	0.18	0.13	0.15	0.03

[a] Dogs BM2251 and BM2252 were male and dogs BF2254 and BF2256 were female.

Table 4. Levels of Radioactivity in Plasma following PO Administration of [^{14}C]Artelinic Acid to Dogs[a]

Sample Time (hr)	BM2251 ugeq/mL	BM2252 ugeq/mL	BF2254 ugeq/mL	BF2256 ugeq/mL	Mean ugeq/mL	S.D.
0.5	8.5	11.6	12.3	10.8	10.8	1.7
1	7.0	6.5	9.2	7.7	7.6	1.2
2	4.5	4.0	5.9	3.9	4.6	0.9
4	5.0	2.7	3.7	2.6	3.5	1.1
8	3.5	2.5	3.4	3.0	3.1	0.5
12	2.5	2.8	2.5	1.7	2.4	0.5
24	1.1	2.1	1.4	0.78	1.4	0.6
48	0.36	0.50	0.54	0.38	0.44	0.1
72	0.27	0.27	0.41	0.25	0.30	0.1
96	0.23	0.22	0.33	0.21	0.25	0.1
120	0.18	0.18	0.26	0.19	0.20	0.04
144	0.16	0.17	0.22	0.16	0.18	0.03
192	0.14	0.14	0.20	0.11	0.15	0.04

[a] Dogs BM2251 and BM2252 were male and dogs BF2254 and BF2256 were female.

Table 5. Summary of the Urinary and Fecal Elimination of Radioactivity after IV or PO Administration of [^{14}C]Artelinic Acid to Dogs

		% of Dose			
Dog	Dose Route	Urine	Rinse	Feces	Total
AM2249	IV	23.5	11.6	57.5	92.6
AM2250	IV	44.1	7.6	41.7	93.4
AF2253	IV	41.4	7.9	45.2	94.5
AF2255	IV	39.7	7.9	44.7	92.3
Mean		37.2	8.8	47.3	93.2
BM2251	PO	24.8	8.4	60.1	93.3
BM2252	PO	25.9	11.5	53.4	90.8
BF2254	PO	18.5	17.4	57.2	93.1
BF2256	PO	32.7	2.3	59.2	94.2
Mean		25.5	9.9	57.5	92.9

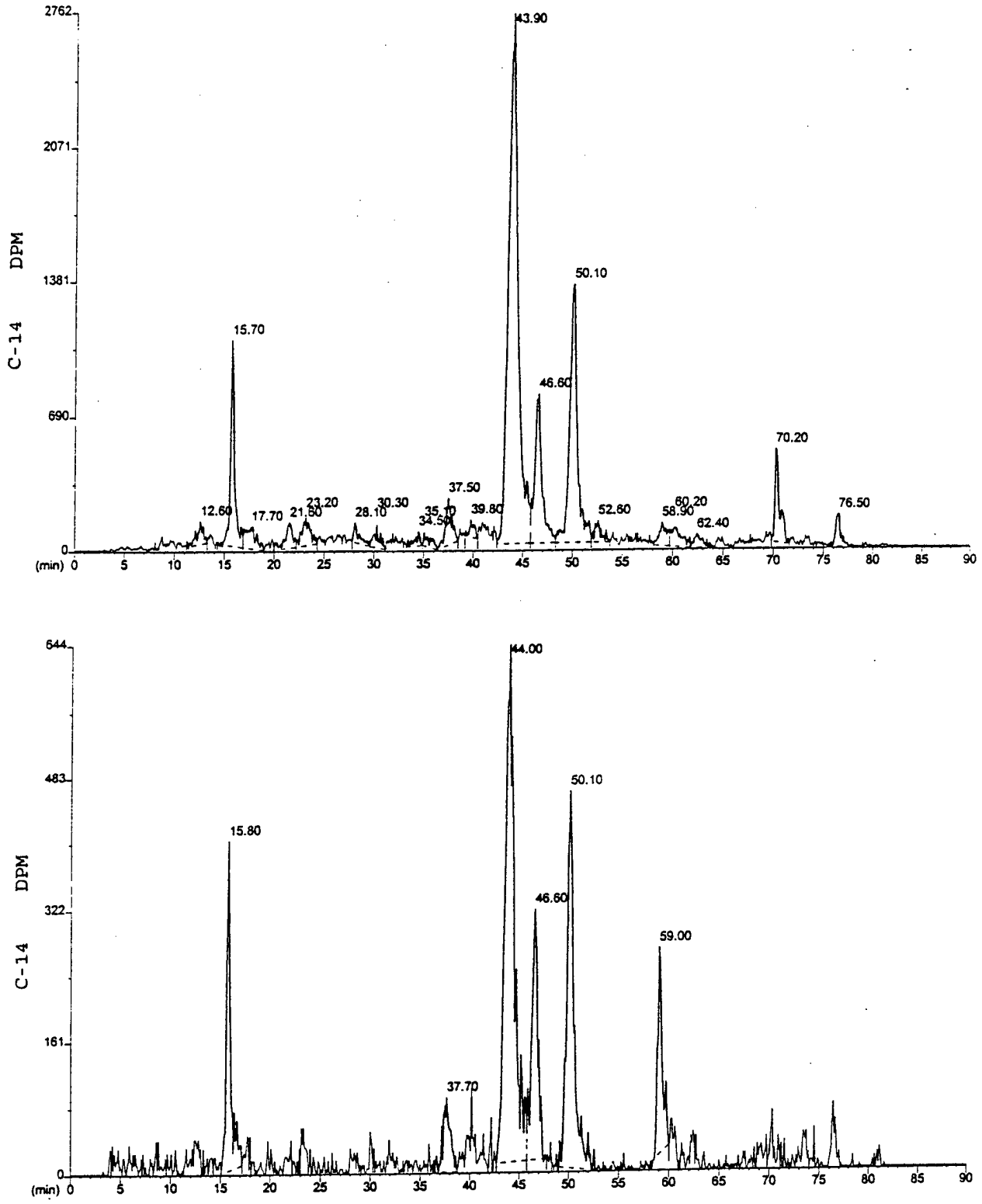

Figure 1: Elution profile of radioactivity obtained during the HPLC analysis of urine collected 0-12 hours (upper panel) or 12-24 hours (lower panel) after IV administration of [^{14}C]artelinic acid to a dog. In this system, unchanged artelinic acid had a retention time of approximately 85 minutes.

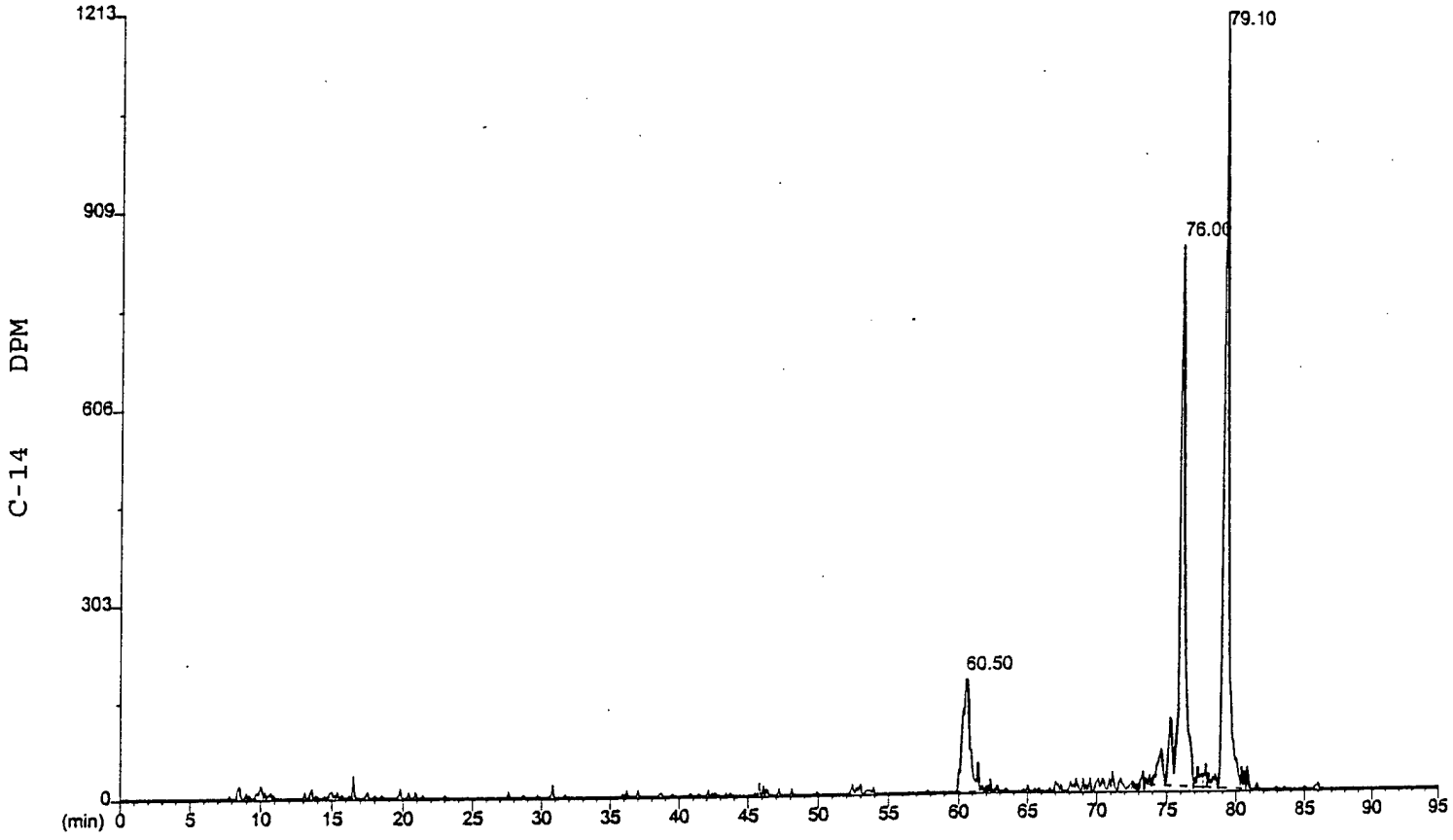

Figure 2: Elution profile of radioactivity obtained during the HPLC analysis of feces collected from 12-24 hours after IV administration of [^{14}C]artelinic acid to a dog. In this system, unchanged artelinic acid had a retention time of approximately 85 minutes.

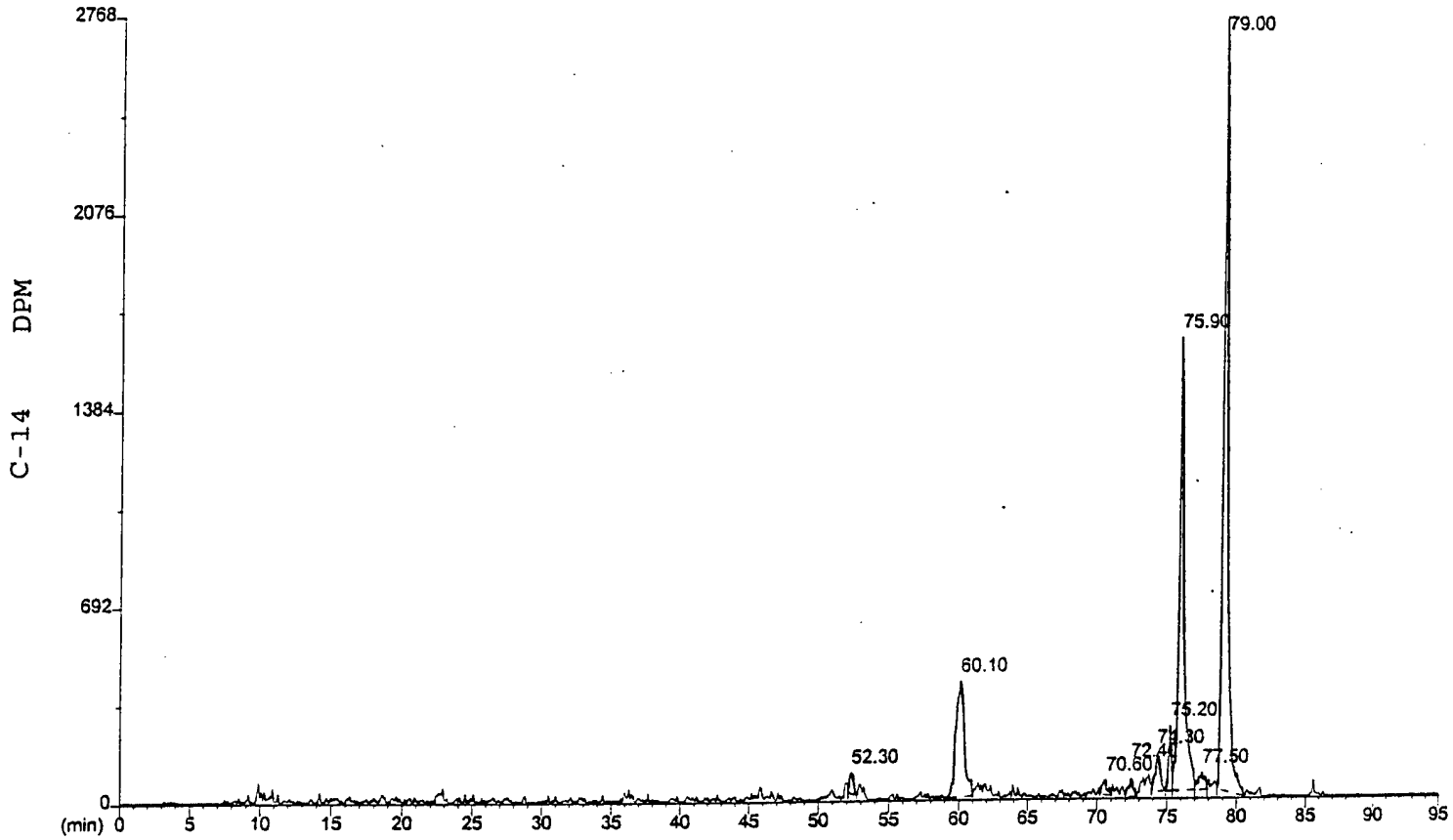

Figure 3: Elution profile of radioactivity obtained during the HPLC analysis of feces collected from 12-24 hours after administration of [^{14}C]artelinic acid to a dog. In this system, unchanged artelinic acid had a retention time of approximately 85 minutes.